THE GREATER OMENTUM AS A SITE OF ANTIBODY SYNTHESIS

F. C. WALKER AND A. W. ROGERS

From the Departments of Surgery and Anatomy, The Medical School, Birmingham 15

Received for publication November 24, 1960

THE greater omentum is a structure which has long excited the interest of surgeons and anatomists, and many theories have been advanced about its function. From clinical and post-mortem evidence, it is clear that the omentum plays an important role in surrounding and localising intraperitoneal foci of infection. The suggestion has also been made by Portis (1919) and Roberts (1955) that the omentum is an important site of antibody formation when the route of administration of antigen is intraperitoneal.

Walker, Thomson and Gray (1960) have reviewed the clinical and experimental work on this subject, and have described a series of studies designed to investigate the immunological potential of the omentum. Using rabbits immunized against *Salmonella typhi* by a course of intra-peritoneal injections, they showed that the titre of specific antibody in the omentum was higher than the serum titre, and higher than the titres of spleen, liver and peritoneum. In an animal from which the greater omentum had been removed surgically before immunization, the serum antibody level was considerably lower than that seen in intact animals. Portions of omentum from immunized animals were taken for culture *in vitro*, and the medium was found to contain measurable titres of specific antibody. Similarly, homotransplants of portions of omentum to non-immune recipients resulted in a serum antibody response which differed in pattern from that seen after the transfer of immune serum, or from the normal course of a primary immune response. Using the fluorescent antibody technique of Coons, Leduc and Connolly (1953), antibody-containing cells were demonstrated amongst the many lymphocytes and plasma cells which appeared in the omentum during immunization.

These findings provide powerful support for the suggestion that the greater omentum is an active site of antibody synthesis following intraperitoneal immunization. The experiments to be described here were designed to confirm these observations, and to provide direct evidence that the high antibody titres associated with the greater omentum are due, at least in part, to antibody synthesis by that tissue. To do this, it is necessary to show that the serum antibody titres seen in non-immune animals receiving grafts of immune omentum are not the result of the transfer of preformed extracellular antibody trapped in the tissue spaces of the graft, nor due to the release of intracellular antibody also present at the time of transfer. Preformed antibody might have originated in some other tissue, and its presence in the omentum could be the result of the inflammation caused by the intra-peritoneal injections of antigen.

Keston and Katchen (1956) and Humphrey and Sulitzeanu (1957) have used radio-isotope techniques with this same objective in view in studies of antibody

synthesis by tissue explants cultured *in vitro*, while Stavitsky (1956) employed similar methods in demonstrating antibody synthesis by lymph node cells transplanted into recipient animals. Humphrey and Sulitzeanu (1957) examined the time relations of antibody synthesis in various tissues of the rabbit. In order to measure the amount of preformed extracellular antibody carried over into the culture medium with the explant, the rabbits were injected with serum containing high titres of the specific antibody. This serum had been labelled with ^{131}I, and was injected 2–3 days before the explants were removed, to allow time for the labelled antibody to come into equilibrium with the extracellular antibody already present in the animal. The radioactivity of the antibody precipitated from the culture medium gave a direct measure of the extent to which preformed extracellular material had contributed to the precipitate. Active antibody synthesis *in vitro* was demonstrated by adding ^{14}C-labelled amino-acids to the culture medium, and estimating the radioactivity of the antibody subsequently isolated.

The present experiments followed this general design, with homografts of immune omentum to non-immune recipients replacing the explants into tissue culture. Recovery of specific antibody from the serum of the recipient was necessary in order to make the estimates of radioactivity, so a system giving precipitation of antibody with antigen was used. Pneumococcal capsular polysaccharide was chosen as the antigen, and the opportunity taken to repeat some of the experiments of Walker *et al.* (1960), which employed *Salm. typhi.*

The counting equipment available to us at the time of the experiment was not sensitive enough to give valid results with the low activities obtained. Autoradiographic emulsions have a high efficiency for low energy B-emitting isotopes such as ^{14}C, and long exposure periods make it possible to record very low activities at a level which is statistically useful. Autoradiography was therefore used to determine the levels of radioactivity found in the antigen-antibody precipitates.

MATERIALS AND METHODS

Adult white and sandy-lop rabbits were used. These were bred at the National Institute for Medical Research, Mill Hill, and at the Department of Experimental Pathology, Birmingham University, and were maintained on pelleted diet No. 18 (Bruce and Parkes, 1940) supplemented with hay.

Throughout the transplantation experiments, sandy-lop animals were used as donors, and whites as recipients. The serum antibody titres in normal, immunized animals were studied in 3 sandy-lop rabbits, and the serum titres in animals from which the greater omentum was excised surgically before the course of immunizing injections in a further 2 sandy-lops. Two donor rabbits and 2 recipients were used in the radio-isotope studies.

Formalin-killed type III pneumococci were obtained from the Standards Laboratory, Colindale, for the immunizing injections, and for the preparation of purified capsular polysaccharide by acid hydrolysis. The immunization consisted of six intra-peritoneal injections given at 3 day intervals. Each injection comprised 1 ml. of a suspension of pneumococci standardised to give an optical density equivalent to that of a No. 7 Brown's opacity tube.

Serum antibody was precipitated by the stepwise addition of small amounts of purified antigen until no further precipitation occurred. With this particular system, precipitation is not inhibited by a two- to three-fold excess of antigen, so that this procedure is unlikely to result in loss of antibody. After the final addition of antigen, the samples were left overnight at 20° for the deposition of antibody to continue to completion.

Procedure for radio-isotope experiments

A control, non-radioactive, antigen-antibody system was used to measure the level of radioactivity adsorbed upon a precipitate formed in serum containing labelled material.

Purified ovalbumin was used as the precipitating antigen. Dried rabbit anti-ovalbumin was available in the Department of Experimental Pathology. Each recipient animal was given an intravenous injection of 500 mg. of this, dissolved in 5 ml. of distilled water.

Rabbit serum containing a high titre of anti-pneumococcal (type III) antibody was labelled with ^{131}I by the rapid mixing method of McFarlane (1956). Each donor animal received 55 μc. of this labelled serum intravenously.

An acid hydrolysate of Chlorella protein, uniformly labelled with ^{14}C, was obtained from the Radiochemical Centre, Amersham, and 100 μc. administered intraperitoneally to each recipient rabbit.

On the 16th day after the first of the series of immunizing injections, the two donor rabbits were given sodium iodide in their drinking water, at a concentration of 50 mg./l. This was continued up to the time of transplantation, to reduce to a minimum the thyroidal uptake of radio-iodide liberated by the breakdown of the ^{131}I-labelled serum. On the 18th day, each donor rabbit was given 55 μc. of ^{131}I-labelled serum, intravenously. On the 20th day, the rabbits were killed. The thorax was opened immediately, the right auricle removed, and a cannula placed through the interatrial septum into the left atrium. Two litres of normal saline were perfused into the left atrium, and thence throughout the body, using the heart as a manual pump. This cleared most of the blood from the tissues, minimising the chance of including antibody-producing cells from the blood in the transplanted omentum, and removing the intravascular component of any preformed extracellular antibody. The greater omentum was then excised and weighed, and 7 g. transferred immediately to the abdominal cavity of a non-immune recipient rabbit, as a free, intraperitoneal graft.

Several hours after the transplantation, each recipient animal received 500 mg. of anti-ovalbumin intravenously. Blood samples were taken from each recipient for the estimation of the level of radioactivity in the specific antibody 48 hr. after transplantation. Any activity found in this sample could only be attributed to the ^{131}I-labelled serum, and would indicate a transfer of extracellular antibody in the graft. Immediately after withdrawing this sample, the two rabbits were injected intraperitoneally with 100 μc. each of ^{14}C-labelled amino acids. Further blood samples were withdrawn for autoradiography 8 hr., 5 days, and 7 days after the injection of labelled amino acids.

Procedures for autoradiography

Two areas $\frac{3}{4}$ in. square were marked with a diamond pencil on each of a number of microscope slides, and labelled A and C. The slides were carefully cleaned, and then dipped in a dilute aqueous solution of gelatin (gelatin, 5 g., chrome alum, 0·5 g., water to 1 l.), and allowed to dry.

The serum from each blood sample was divided into two equal portions. Pneumococcal capsular polysaccharide was added to the first until precipitation was complete, and ovalbumin to the second. The resultant precipitates were carfeully washed, and redissolved in 0·5 ml. each of distilled water. 0·1 ml. of this solution from the pneumococcal system was placed on the square marked A on the appropriate slide, and spread to cover the entire square. A similar amount of the control solution containing anti-ovalbumin was placed in the square marked C. Both were evaporated to dryness at room temperature. Each slide therefore carried the specific, endogenous, and the control, exogenous, antigen-antibody precipitates, derived from the same volume of serum.

Autoradiographs of these slides were prepared using Kodak AR–10 stripping film, as described by Pelc (1956), and also by applying Ilford K2 nuclear research emulsion by the painting technique of Belanger (1950). The slides from the first blood sample were prepared within 2 days of taking the sample. Both the AR–10 and the K2 autoradiographs of this sample were exposed for 28 days.

The slides from the subsequent blood samples were left for about 10 weeks before autoradiographs were prepared, to allow any ^{131}I activity initially present to decay away to insignificant levels. The AR–10 autoradiographs from these samples were exposed for 14 days, the K2 autoradiographs for 18 days. After development and fixation, they were mounted under coverslips for microscopy.

Grain counts were carried out on a photometric device to be described fully elsewhere (Rogers, unpublished). This employs a photomultiplier tube mounted on a microscope, which is fitted for incident illumination. The light output from each field viewed in this way is made up of two components : light reflected and scattered by the optical system, which

is constant for different fields on the same slide, and light reflected by the grains of metallic silver in the photographic emulsion. Above a threshold value, which represents the first component, the response of the machine increases linearly with increasing number of silver grains. Any difference in the observed light output between fields in the A and C squares of the same slide is due entirely to a difference in grain density. The readings are in the form of a galvanometer deflection, and are expressed in arbitrary units. To avoid bias in the selection of fields for measurement, the readings in each square were taken in 5 or 6 rows of 6 readings each. Two millimetres separated each reading from the next, and each row from the next. These readings have proved more accurately reproducible, and far quicker to take, than visual grain counts. The readings taken from one of the slides were checked against a series of visual grain counts.

The AR–10 stripping film is uniform in thickness over the entire slide, and it was expected that this would make it more suitable for quantitative work than the K2 emulsion. In fact, the photometric readings were much more variable, and, in general, higher with the stripping film. This confirmed the impression obtained visually that the grain densities were much more variable than with the painting technique. This high " background " of developed silver grains was presumably due to the greater degree of manipulation required in stripping film, and was sufficient to make accurate observations difficult with some slides. Table I

TABLE I.—*Photometric Readings Taken from K2 and AR–10 Autoradiographs of Precipitates from the Same Blood Sample, and Visual Grain Counts from a Different Series of Fields on the Same Ar–10 Slide*

Slide		Estimations of relative grain density				
		Mean	Number of observations	Standard deviation	Standard error	*P**
K2 slide	Square A	7·23	36	3·73	0·621	
	Square C	5·43	36	1·48	0·246	0·01–0·001
AR–10 slide—	Square A	6·43	30	6·03	1·100	
Photometric readings	Sauare C	3·04	30	2·80	0·510	0·01–0·001
AR–10 slide—	Square A	145·0	18	73·59	17·34	
Visual grain counts	Square C	102·0	18	53·17	12·53	0·50–0·02

* The statistic P was derived by Student's t-test.

compares the photometric measurements from a K2 slide, similar measurements from an AR–10 slide, and a series of visual grain counts from the latter slide. Both slides were of the same antigen-antibody precipitate, that from the blood sample taken 8 hr. after the administration of ^{14}C-labelled amino acids to one of the recipient rabbits. It will be seen that the photometric readings from the AR–10 slide had a higher standard deviation than those from the K2 slide. Both the series of photometric readings agreed well in showing a significantly higher mean reading in the A square than in the C square. This close agreement between the results with the two different techniques suggested that no gross error was introduced by the inevitable slight variations in thickness in the K2 emulsion. The visual grain counts, which were all carried out by the same observer, were from larger fields than the photometric readings. Approximately the same total area of emulsion was scanned by the two methods. The visual counts confirmed that the difference in photometric readings between the A and C squares corresponded to a difference in grain density.

In view of the high and unpredictable level of " background " found with the AR–10 slides, the results will be based on the K2 series of autoradiographs.

RESULTS

Table II shows the serum antibody titres of 3 normal rabbits during the course of immunizing injections, and the serum titres of 2 rabbits from which the greater omentum had been excised prior to the immunizing injections. It will be seen that the animals which lacked the omentum responded with lower serum titres to the intraperitoneal injection of antigen.

TABLE II.—*Serum Antibody Titres of 5 Rabbits Given a Course of Intraperitoneal Injections of Type III Pneumococci*

Rabbit	Days after first immunizing injection			
	5	10	15	20
1. Intact . . .	1 : 200	1 : 400	1 : 800	1 : 3200
2. „ . . .	1 : 50	1 : 100	1 : 800	1 : 1600
3. „ . . .	1 : 100	1 : 400	1 : 3200	1 : 6400
4. Without omentum .	1 : 50	1 : 50	1 : 100	1 : 800
5. „ „ .	1 : 50	1 : 100	1 : 400	1 : 400

Fig. 1 shows the serum antibody titres found in a non-immune recipient rabbit following transplantation of omentum from an immune donor. A measurable serum titre appeared within 6 hr., and this rose to a high level until between the 9th and 11th days when a sharp drop occurred. It seems reasonable to assume that destruction of the grafted omentum by the immune reaction of the host was responsible for this fall in antibody titre.

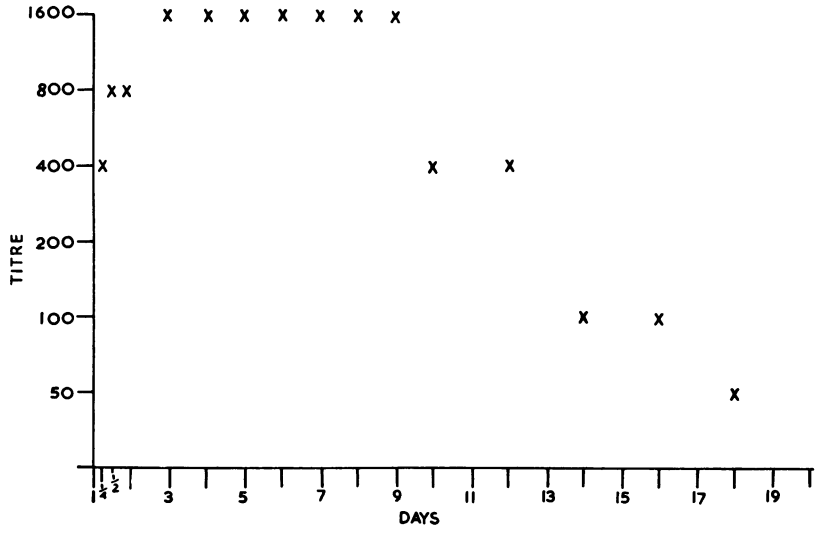

FIG. 1.—The serum titres of specific antibody in a non-immune rabbit that received an intraperitoneal graft of omentum from an immunized donor on day 1.

Table III presents the data obtained from the K2 autoradiographs derived from the blood samples of the first recipient animal. The means of the photometric readings in this series are shown graphically in Fig. 2.

Taking the results from the control, ovalbumin, system first, it will be seen that the readings from the samples obtained before the administration of ^{14}C-labelled amino-acids, and those 5 and 7 days later, were uniformly low. Student's *t*-test fails to show any significant difference between these figures. By contrast, the sample taken 8 hr. after the administration of ^{14}C gave a higher reading. Evidently, the precipitate which formed in the serum at this time carried down with it adsorbed radio-active material which was not removed by subsequent washing. Many of the labelled compounds present in the serum at this time were

TABLE III.—*Photometric Readings from Autoradiographs of Serum Samples from a Non-immune Rabbit Grafted with Immune Donor Omentum*

Slide		Estimations of relative grain density: Mean	Number of observations	Standard deviation	Standard Error	*P*
First sample—	Square A*	1·19	36	0·409	0·068	C > A
before injection of ^{14}C	Square C	1·66	36	0·684	0·114	<0·001
Second sample—	Square A	7·23	36	3·73	0·621	A > C
8 hr. after injection of ^{14}C	Square C	5·43	36	1·48	0·246	<0·01, >0·001
Third sample—	Square A	4·41	36	3·22	0·537	A > C
5 days after injection	Square C	1·85	36	1·29	0·215	<0·001
Fourth sample—	Square A	2·19	30	0·837	0·153	A > C
7 days after injection	Square C	1·91	30	0·496	0·091	0·2–0·1

* The specific antibody was in Square A, the control antibody in Square C.

presumably either catabolised and excreted, or incorporated into intracellular material by the time the 5 day sample was taken.

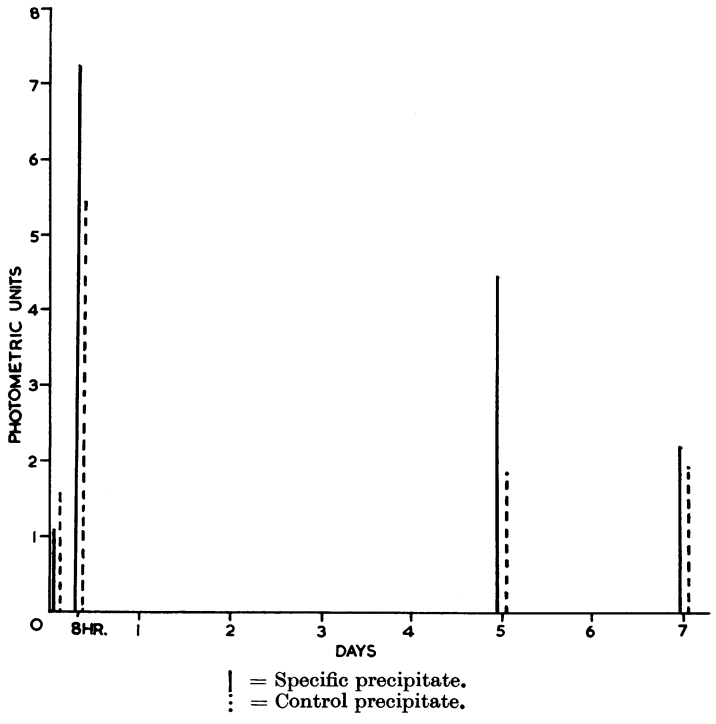

FIG. 2.—The means of the photometric readings from the K2 series of autoradiographs prepared from the serum samples of the first recipient animal. The first sample was taken after transplantation of omental tissue, and immediately before the injection of ^{14}C-amino acids.

Turning next to the readings obtained from the specific (pneumococcal) system, those from the first sample were lower than those for the control system. There was therefore no radioactivity in this precipitate which could be attributed to the ^{131}I-labelled serum given to the donor animal. Eight hours after the ad-

ministration of ^{14}C amino acids, the readings over the specific precipitate were higher than those over the control. This difference is statistically significant by Student's t-test ($P < 0{\cdot}01$, $> 0{\cdot}001$). In the autoradiographs of the 5 day sample, this difference was even more marked ($P < 0{\cdot}001$). By 7 days, readings over both precipitates were again equal.

The results from the second recipient rabbit were not quite so satisfactory, but they confirmed the general pattern seen in the first animal. Before the administration of ^{14}C amino acids, there was no significant difference between specific and control systems ($P < 0{\cdot}9$, $> 0{\cdot}8$). Eight hours later, the readings from the autoradiograph of the specific precipitate were higher ($P < 0{\cdot}1$, $> 0{\cdot}05$). By 5 days, this difference had become statistically significant ($P < 0{\cdot}01$, $> 0{\cdot}001$). At 7 days there was again virtually no difference between the two ($P < 0{\cdot}2$, $> 0{\cdot}1$).

DISCUSSION

The results presented here, utilising pneumococcal capsular polysaccharide as antigen, confirm the general pattern found by Walker *et al.* (1960) with *Salm. typhi*. The removal of the greater omentum before a course of intraperitoneal injections of antigen results in lower serum antibody levels than are seen in the intact animal. Transplantation of a portion of omentum from a donor immunized in this way to a non-immune recipient is followed by the appearance of specific antibody in moderately high titre in the serum of the recipient. But this observation may be interpreted in three ways. The antibody found in the recipient may result from the transfer of antigen in the graft, with subsequent antibody production by the host. The observed serum titres may be due to the presence of preformed antibody, either extracellular or intracellular, in the grafted tissue. Finally, the graft may actively synthesize specific antibody in the non-immune host.

Several workers have used this experimental pattern of the transfer of cells from an immune donor into a non-immune recipient, in order to investigate possible sites of antibody synthesis. Harris, Harris and Farber (1954), for example transferred popliteal lymph node cells from rabbits that had been inoculated in the corresponding foot, and found an antibody response in the recipient. Stavitsky (1956) found a similar antibody response after the transfer of lymph node and spleen cells. Oakley, Warrack and Batty (1954) transplanted a number of tissues, and made a careful study of the time relations of the resulting antibody response. In all three groups of experiments, the pattern of antibody formation resembled that illustrated in Fig. 1. Demonstrable antibody appeared far sooner than would be expected in a primary response. Oakley *et al.* were able to show a typical primary response by the host animal in experiments which favoured the persistence of antigen in the transplant. When this was seen, it did not occur until about 10 days after the transplantation, and could be clearly distinguished from the earlier antibody response attributed to the graft itself. In the present experiments, no such diphasic response was seen. Presumably the high antibody titres in the donor at the time of transplantation made it unlikely that antigen would persist in significant amounts. It seems very improbable then, that antibody formation by the host animal could account for the pattern of response found in these experiments.

Both Oakley *et al.* (1954) and Walker *et al.* (1960) studied the serum antibody titres in the non-immune recipient after the injection of serum from an immune

donor. In this type of experiment, where preformed extracellular antibody is deliberately transferred in considerable amounts, an initial antibody peak is found in the serum of the recipient, followed by a rapid exponential drop during the subsequent few days. The persistence of a high antibody titre in the recipient for 10 days, as in the experiments reported here, cannot be explained as the transfer of preformed extracellular antibody. This view is consistent with the failure to detect any radioactivity in the first serum samples from the recipient animals.

The possibility that the antibody titres seen in the recipients were due to the slow release of preformed, intracellular, antibody by the graft is also remote. Walker *et al.* heated portions of omentum, before placing them in the recipient, to a temperature sufficient to kill the cells, but not high enough to denature any preformed antibody present. The serum titres in the recipient showed a low peak 1–2 days later, falling away to zero in 5–6 days. This pattern, too, fails to correspond to that seen following grafts of living omentum. It may be argued that, in the latter case, when cells were transferred live, the process of lysis and liberation of intracellular antibody might have followed a different time course. In such a graft, however, many of the cells presumably would remain alive until about the 10th day, when widespread cell destruction would accompany the reaction of the host against the homograft. If the antibody circulating in the recipient had been preformed in the donor, and intracellular in location, a rise in serum titre might have been expected at this time of increased cell death. But the observed titres started to fall at this time, suggesting that the presence of live cells from the donor was necessary to maintain the antibody titres seen in the recipient.

It therefore seems highly unlikely that preformed antibody either extracellular or intracellular, contributed significantly to the serum titres of the recipients. On the other hand, the incorporation of ^{14}C amino acids into the antigen-antibody precipitates showed conclusively that molecules of specific antibody had been synthesised in the recipient animals. Stavitsky (1958) in a similar type of experiment, was able to demonstrate the incorporation of ^{35}S methionine into specific antibody in the non-immune recipient. It is interesting to note that, in the experiments reported here, there was no detectable radioactivity in the antigen-antibody precipitates 7 days after the injection of ^{14}C amino acids, although the serum titre of antibody remained high until after the 10th day. Humphrey and McFarlane (1954) have shown that isotopically labelled antibody molecules have a biological half-life in the rabbit of about 120 hr. In the light of this, it seems reasonable to interpret the results as demonstrating the loss by catabolism and excretion of the molecules of radioactive antibody over the course of 7 days, and their replacement by a population of antibody molecules synthesized after labelled amino-acids ceased to be available. It is difficult to avoid the conclusion that the greater omentum is the site of active antibody formation when the antigen is administered into the peritoneal cavity.

The sites of antibody synthesis fall into two main groups. The first comprises the regional lymph nodes, spleen, lungs, and bone marrow, in which active synthesis may take place in response to an antigen injected elsewhere in the body. Humphrey and Sulitzeanu (1957) and Askonas and Humphrey (1958) have shown that, in the rabbit, an intravenous injection of antigen is followed by antibody production in the spleen, lungs and bone marrow, in descending order of magni-

tude. The subcutaneous injection of antigen results in antibody synthesis in the regional lymph node (Harris *et al.*, 1954 ; Coons *et al.*, 1953 ; Oakley *et al.*, 1949). The second group consists of certain tissues which respond to the local injection of antigen by the local formation of antibody. Askonas and Humphrey, for example, were able to show that the granuloma which develops at the site of intramuscular injection of antigen mixed with Freund's adjuvant is the major source of antibody in this instance. Oakley, Batty and Warrack (1951) and Oakley *et al.* (1954) demonstrated local antibody synthesis in fat from several parts of the body, including the omentum, skin, and skeletal muscle. These three tissues appeared to retain antigen longer than the other tissues studied at the same time, as judged by their ability to cause a primary response on transplantation into a non-immune host. It is interesting to note that adipose tissue appeared to be incapable of antibody synthesis until 24 hr. after the injection of antigen. Oakley *et al.* related this change in immunological ability to the infiltration of cells around the injection site that accompanied early stages in the formation of the granuloma.

The greater omentum resembles both groups of antibody-forming tissues. It can respond to the injection of antigen directly into its substance by the local synthesis of antibody. It also synthesizes antibody when the antigen is administered into the peritoneal cavity.

SUMMARY

Serum antibody titres were studied in rabbits given a series of intraperitoneal injections of type III pneumococci. The titres were lower in animals from which the greater omentum had been removed than in intact animals.

Portions of the greater omentum were transplanted from rabbits immunized by the intraperitoneal route into the abdominal cavities of non-immune recipients. A characteristic rise and fall in serum antibody titre followed, suggestive of active antibody synthesis by the graft within the host animal.

Rabbit serum containing the specific anti-pneumococcal antibody was labelled with ^{131}I, and administered to immune donor animals before transplantation of portions of omentum to non-immune recipients. Autoradiographic examination of the serum antibody found later in the recipients failed to detect any radioactivity.

^{14}C-labelled amino-acids were administered to non-immune rabbits that had received intraperitoneal grafts of omentum from immunized donors. Autoradiographic examination of the specific antibody found in the serum of the recipients showed that it contained significant levels of radioactivity 8 hr. and 5 days later.

These findings are discussed, and it is concluded that the greater omentum is an important site of active antibody synthesis following the intraperitoneal injection of antigen.

The authors wish to express their gratitude to Dr. J. H. Humphrey for advice on the design of these experiments, to Dr. J. Davies for labelling rabbit serum proteins with ^{131}I, and to Dr. E. H. Ashton for guidance with the statistical material. Professor J. R. Squire and Professor P. G. H. Gell gave valuable advice and criticism. The authors also wish to thank Miss N. Dews for skilled technical assistance.

REFERENCES

ASKONAS, B. A. AND HUMPHREY, J. H.—(1958) *Biochem. J.*, **68**, 252.
BELANGER, L. F.—(1950) *Anat. Rec.*, **107**, 149.
BRUCE, H. M. AND PARKES, A. S.—(1940) *J. Hyg., Camb.*, **44**, 501.
COONS, A. H., LEDUC, E. H. AND CONNOLLY, J. M.—(1953) *Fed. Proc.*, **12**, 439.
HARRIS, S., HARRIS, T. N. AND FARBER, M. B.—(1954) *J. Immunol.*, **72**, 148.
HUMPHREY, J. H. AND MCFARLANE, A. S.—(1954) *Biochem. J.*, **57**, 186.
Idem AND SULITZEANU, B. D.—(1957) *Ibid.*, **68**, 145.
KESTON, A. S. AND KATCHEN, B.—(1956) *J. Immunol.*, **76**, 253.
MCFARLANE, A. S.—(1956) *Biochem. J.*, **62**, 135.
OAKLEY, C. L., BATTY, I. AND WARRACK, G. H.—(1951) *J. Path. Bact.*, **63**, 33.
Idem, WARRACK, G. H. AND BATTY, I.—(1949) *Ibid.*, **61**, 179.—(1954) *Ibid.*, **67**, 485.
PELC, S. R.—(1956) *Int. J. app. Rad. and Isotopes*, **1**, 172.
PORTIS, B.—(1919) *Trans. Chicago path. Soc.*, **11**, 399.
ROBERTS, K. B.—(1955) *Brit. J. exp. Path.*, **36**, 199.
STAVITSKY, A. B.—(1956) *Fed. Proc.*, **15**, 615.—(1958) *Brit. J. exp. Path.*, **39**, 46.
WALKER, F. C., THOMSON, J. D. AND GRAY, J. G.—(1960) *Brit. J. Surg.*, **48**, 89.